Remarks and Arguments

After having been allowed, and the issue fee paid, the present application was withdrawn from issue and subjected to further evaluation, which resulted in the currently outstanding office action, in response to which this amendment is being filed. During the original prosecution stages, two office actions were issued by the examiner, and two responses were filed by the applicants, along with a terminal disclaimer, that ultimately led to the application's allowance. However, new prior art has now been cited, and each of the previously-allowed claims has now been rejected. Those rejections are discussed in more detail below.

Claims 1-23 were rejected under 35 U.S.C. §103(a) as being obvious over U.S. Patent No. 6,221,601 ("Koster '601) in view of either U.S. Patent No. 5,547,835 ("Koster '835) or U.S. Patent No. 5,830,655 ("Monforte"). In making this rejection, the examiner has stated that Koster '601 discloses providing an extension primer having a nucleotide chain that contains a cleavable linker and attaching the primer to DNA adjacent to a mutation site, extending the primer using mutation dependent primer extension, cleaving the linker to produce a DNA cleavage product and analyzing the cleavage product using mass spectrometric analysis. That is, the examiner repeats all of the language of the applicant's Claim 1, except for the limitation regarding the use of a photocleavable linker. The examiner states that Koster '601 does not disclose a photocleavable linker, and therefore cites Koster "835 and Monforte. However, there are distinctions between the present invention and the prior art combinations proposed by the examiner that are not taken into account by the outstanding prior art rejection.

Koster '601 discloses a method of DNA detection using mass spectrometry. Koster '601 discusses different embodiments for doing the DNA detection, and mentions the use of a cleavable linker. Strangely, in the section beginning in column 4 that was identified by the examiner, and in example 7, there is no mention of using a primer that has a cleavable linker. However, the examiner is correct that Claim 1 mentions cleaving the primer from the extension product. Nevertheless, Koster '601 is distinctly different from the present invention. Koster '601 uses a cleavage site that is

located at the 5' end of the primer. The apparent reason for this is to allow the primer and extension products to be released after being immobilized by attachment to a solid support. Cleavage would then allow measurement of the cleaved primer/extension products by mass spectrometry. However, Koster is unconcerned with limiting the number of fragments of different lengths, or controlling the length of the fragments for improving their measurement by mass spectrometry.

The Koster '835 reference was cited as disclosing the use of a primer with a photocleavable linker. Koster '835 discusses a method for determining a DNA sequence that uses the Sanger method of detecting the nested fragments obtained by base-specific chain termination. As noted in column 10, lines 8-11, Sanger sequencing produces four families of chain-terminated fragments. Koster uses the molecular weight of the fragments, as determined by mass spectrometry, to catalog the families for the purpose of sequencing. The method may include the use of a primer having a cleavable linker at the 5' end that allows attachment to a solid support. When attached, the extension product may be washed to remove all of the reaction by-products. The nested fragments are then cleaved off the solid support to allow measurement by mass spectrometry. As noted by the examiner, the cleavable bond may be a photocleavable bond. Koster '835 is, however, focused on sequencing, and appears unrelated to mutation analysis.

Monforte, like Koster '835, is directed to DNA sequencing, and provides a method for improving the read length of the fragments generated during the step of base-specific primer extension. The standard method of generating sequence ladders is used as a method of identifying the content of a target DNA molecule. As with Koster '835, immobilization to a solid support is used to allow washing of the extension products prior to using a detection method such as mass spectrometry. As noted by the examiner, Monforte also mentions the possible use of photocleavable linkers in the primers applied in the method. Monforte additionally suggests locating the linker near the 3' end of the primer to improve the "read length" of the extension segments generated. In Example 7, the method of Monforte is applied for the purposes of

detecting a point mutation and, as shown in the accompanying Figures 14A and 14B, the spectra derived from the extension and analysis of two different target molecules may be compared to identify a difference in the separation in certain mass peaks of the two spectra, indicating a mutation in at least one of the bases. However, with the extension method used, multiple fragments are generated for each target molecule.

The present invention is directed to mutation analysis that uses modified primers in a primer extension that is terminated at the same point in all cases for a given target molecule, thereby resulting in fragments all having the same length. The primer includes a photocleavable linker that is positioned within a few nucleotides of the 3' end of the primer. After mutation-dependent extension of the primer, the linker is cleaved and short fragments of the extended primer are generated which serve as products for the mass spectrometric measurements. These short fragments contain the desired information regarding the mutation. The length of all the fragments derived from one reaction is the same. The reaction products for different alleles differ in their molecular weight depending on the kind of mutation. The determination of the mass of all fragments with the same length yields the correct genotype which has to be determined.

As the question of the analysis is to determine the nucleotide which is present at a potential mutation site of a known nucleic acid molecule, the length of the cleavage fragment can be selected in advance. Unlike the Sanger sequencing techniques used by Koster '835 and Monforte (which are intended to generate a multiplicity of fragment lengths, so as to allow for sequencing of a DNA molecule), the present invention is focused on very precise analysis of a single nucleotide polymorphism within a known sequence. This allows the molecular mass of the fragments to be selected to fall within a small predetermined mass range, which is most suitable for either or both of MALDI (matrix-assisted laser desorption ionization) and ESI (electrospray ionization) mass spectrometers.

The present invention differs significantly from Koster '601, which is unconcerned with the length of the extension product. While Koster '601 discusses a technique for

doing mutation analysis, this technique does not attempt to control the extension length, but relies on the available resolution of the mass spectrometry process to determine the presence of a mutation. In contrast, the present invention locates a photocleavable linker close to the 3' end of the primer, keeping the resulting extension product short, and uses one or more chain terminators to ensure that the resulting fragments are of a constant length, but differing in their molecular weight, the length being one that places the fragment in a mass range that lends itself to good mass spectrometric results. The methods of Koster '835 and Monforte, when combined with Koster '601, do nothing to make the prior art more suggestive of the applicants' invention. Koster '835 discloses a Sanger-type sequencing technique that produces a multiplicity of fragments, none of which are optimized for mass spectrometric detection. Monforte, while discussing locating a linker near the 3' end of the primer to improve the read length, also uses a Sanger-type technique that generates a multiplicity of fragments and provides not control over the fragment lengths in a way that enhances mass spectrometric detection.

In order to more clearly highlight the differences between the applicants' claims and the cited prior art, Claim 1 has been amended to specify that, in extending the primer, at least one chain terminator is used that terminates the extension at a predetermined length. This distinguishes the invention from the cited prior art, which is primarily concerned with DNA sequencing, and provides no suggestion of controlling the extension product at a predetermined length. Each of Claims 2-23 depends ultimately from Claim 1 and, in addition to presenting additional limitations, is therefore equally unsuggested by the cited prior art. Reconsideration of Claims 1-23 under this ground for rejection is respectfully requested.

In light of the foregoing amendments and remarks, it is respectfully requested that all the claims be allowed such that the application may be passed to issue. If it is believed that a telephone conference would help expedite prosecution of the application, the examiner is invited to call the undersigned. The Commissioner is

hereby authorized to charge any additional fees due for the filing of this paper to applicants' attorneys' Deposit Account No. 02-3038.

Respectfully submitted

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